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Review

# Adipose tissue engineering with cells in engineered matrices

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**Abbreviations:** $\alpha_2$Col6, alpha 2 chain of collagen type VI; ABCG2, ATP-binding cassette subfamily G member 2; ADD-1/SREBP-1, adipocyte determination- and differentiation-dependent factor-1/sterol regulatory element-binding protein-1; ALDH, aldehyde dehydrogenase; aP2, fatty acid binding protein; ASC, adipose-derived stem cells; bFGF, basic fibroblast growth factor; BM, bone marrow; ECM, extracellular matrix; EGF, epidermal growth factor; C/EBP, CCAAT/enhancer binding proteins; FAS, fatty acid synthase; FAT, fatty acid translocase; GH, growth hormone; Glut4, glucose transporter-4; GPDH, glycerol-3-phosphate dehydrogenase; HA, hyaluronan; HSL, hormone sensitive lipase; IBMX, isobutylmethylxanthine; IGF-1, insulin-like growth factor-1; LPL, lipoprotein lipase; MSC, mesenchymal stem cells; PAI-1, plasminogen activator inhibitor-1; PDM, placental decellular matrix; PEG, polyethylene glycol; PEGDA, polyethylene glycol diacrylate; PGA, polyglycolic acid; PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic); PPARγ, peroxisome proliferator-activated receptor-γ; PPRE, PPAR response elements; PTFE, polytetrafluoroethylene; RXR, retinoid X receptor; T3, triiodothyronine; TNFα, tumour necrosis factor-α; TZD, thiazolidinedione; vWF, von willebrand factor; VEGF, vascular endothelial cell growth factor; XLHA, cross-linked hyaluronan

**Key words:** tissue engineering, regenerative medicine, adipose tissue, adipose-derived stem cells, adipogenesis, cell culture, scaffolds, cell-biomaterial interactions

**Tissue engineering has shown promise for the development of constructs to facilitate large volume soft tissue augmentation in reconstructive and cosmetic plastic surgery. This article reviews the key progress to date in the field of adipose tissue engineering. In order to effectively design a soft tissue substitute, it is critical to understand the native tissue environment and function. As such, the basic physiology of adipose tissue is described and the process of adipogenesis is discussed. In this article, we have focused on tissue engineering using a cell-seeded scaffold approach, where engineered extracellular matrix substitutes are seeded with exogenous cells that may contribute to the regenerative response. The strengths and limitations of each of the possible cell sources for adipose tissue engineering, including adipose-derived stem cells, are detailed. We briefly highlight some of the results from the major studies to date, involving a range of synthetic and naturally derived scaffolds. While these studies have shown that adipose tissue regeneration is possible, more research is required to develop optimized constructs that will facilitate safe, predictable and long-term augmentation in clinical applications.**

## Introduction

The focus of this review is on adipose engineering using a cell-seeded scaffold approach. Tissue engineering holds promise for the treatment of post-operative, congenital or post-traumatic loss of the subcutaneous fat layer, which can result in scar tissue formation deformity, and a loss of function.[1] Current clinical strategies for soft tissue augmentation primarily involve autologous, allogenic and alloplastic materials.[1] Free fat transfer yields unsatisfactory and unpredictable results, with varying degrees of graft resorption due to a lack of supporting vasculature.[2] Only small defects can be corrected with injected autologous fat, and even these limited applications require repeated treatments to maintain the desired volume.[3] Autologous tissue transfer using vascularized flaps that incorporate skin, fat and muscle, represents the gold standard treatment for large volume reconstruction.[4] These techniques require considerable surgical skill, but can yield far superior results to those obtained with synthetic implants. However, there are high costs in terms of donor site morbidity and deformity, as well as hospitalization and surgical time. Alloplastic and allogenic materials can be associated with immune rejection, allergic reaction, implant migration or resorption, and a failure to integrate into the host tissues.[1,5]

To successfully engineer a soft tissue substitute, it is critical to understand the basic physiology of adipose tissue and the process of adipogenesis. As such, we begin with a brief description of the primary functions and cellular composition of fat, with an emphasis on the adipose-derived stem cell population. Following this, we outline some of the key events and mediators involved in adipogenesis. By understanding the cellular processes involved in fat formation, it is possible to more accurately characterize the cellular response within an engineered scaffold, facilitating the optimization of the construct properties to maximize regeneration. The results from some of the main adipose tissue engineering studies involving cells in scaffolds are highlighted, and a perspective for the future is provided.

## Adipose Tissue

Adipose tissue is a dynamic and multi-functional tissue that is ubiquitous throughout the human body.[6] Fat functions as a specialized

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organ that maintains the energy balance through controlled storage and release. Adipocytes store energy in the form of triglycerides, and accumulate or mobilize triacylglycerol in response to the body's energy requirements.[7,8] Adipose tissue is highly plastic and can adapt to facilitate greater storage through the hypertrophic expansion of terminally-differentiated mature adipocytes, as well as the hyperplastic growth and differentiation of precursor cells present in the stroma.[9,10]

In addition to energy regulation, fat functions in maintaining normal body contours, providing mechanical protection, serving as a storage depot for cholesterol and steroid hormones, and is an important site of estrogen biosynthesis.[11-14] Adipose tissue is also the source of multiple autocrine, paracrine and endocrine factors, many of which are involved in energy regulation and the modulation of insulin sensitivity,[15] including leptin,[16] tumor necrosis factor-α (TNFα),[17] plasminogen activator inhibitor-1 (PAI-1)[18] and adiponectin.[19] However, the complement of secreted factors can induce a range of responses within multiple systems, including the reproductive, immunological and vascular systems.[20]

## Cellular Composition of Adipose Tissue

Adipogenesis, the differentiation of precursor cells into mature, terminally-differentiated adipocytes, occurs throughout the human life cycle and is believed to be the primary cause of increases in body fat.[21] Adipose tissue contains multipotent mesenchymal stem cells (MSC), which can differentiate in culture along the adipogenic, chondrogenic, myogenic, osteogenic and putative neurogenic lineages.[22-26] The term "adipose-derived stem cell" (ASC) is used to refer to the collective population of MSC and more-committed adipose progenitors that are found within the stroma of adipose tissue. When induced to differentiate, ASC accumulate intracellular lipid, increasing in diameter up to 20 times, and ultimately forming the characteristic morphology of mature white adipocytes.[7] Adipose tissue also contains fibroblasts, histiocytes and mast cells, as well as cells associated with the vascular and neural systems.[13,27]

## Adipose-Derived Stem Cell Markers

ASC display a very similar immunophenotype to MSC isolated from bone marrow and skeletal muscle.[28,29] Various studies have yielded differences in the detected expression patterns of some markers, potentially due to cell culture variations and/or donor variability.[30] A low percentage of ASC are positive for the multi-lineage marker STRO-1,[31] and, at early passage, a subset express stem cell markers such as CD90 (Thy-1), CD34, ATP-binding cassette subfamily G member 2 (ABCG2), and aldehyde dehydrogenase (ALDH).[30] Preadipocyte factor-1 (Pref-1), a transmembrane protein that is believed to have a role in regulating differentiation, is an additional marker of the undifferentiated cells.[32-35] Endothelial markers can be detected in samples of freshly isolated stromal cells and early passage ASC. These markers include vascular endothelial growth factor receptor (Flk-1), CD31, CD144 and von Willebrand factor.[30] Some additional proteins expressed by the ASC are highlighted in Table 1.

**Table 1 ASC protein expression[28,47,114]**

| | |
|---|---|
| **Cell adhesion molecules** | Integrin β1 (CD29) |
| | Integrin α4 (CD49d) |
| | Vascular cell adhesion molecule (VCAM; CD106) |
| | Intracellular adhesion molecule-1 (CD54) |
| | Activated leukocyte cell adhesion molecule (ALCAM; CD166) |
| **Receptors** | Hyaluronate receptor (CD44) |
| | Transferrin receptor (CD71) |
| | Insulin receptor |
| | Glucocorticoid receptor |
| | Triiodothyronine (T3) receptor |
| | Retinoic acid receptor |
| **Cytoskeletal proteins** | α-smooth muscle actin |
| | Vimentin |
| **Other** | HLA-ABC (Major histocompatibility complex class I antigen) |

## Adipogenic Factors and Differentiation

There appears to be an inverse relationship between ASC proliferation and adipogenic differentiation.[36] In vitro differentiation is associated with growth arrest and can be induced in serum-free medium supplemented with cytokines that target adipogenic pathways.[37] Serum contains multiple factors, such as TNFα, growth hormone (GH) and epidermal growth factor (EGF), that inhibit lipogenesis.[38,39] The differentiation of human ASC is strongly inhibited in the presence of serum, potentially due to its strong mitogenic effect, which may promote cellular decommitment.[40]

The most common components in adipogenic medium formulations include insulin, triiodothyronine (T3), and glucocorticoids.[41,42] Biotin and pantothenate are added to facilitate lipogenesis from glucose.[37] Isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor that has a protective effect on cAMP, has been included in some formulations with positive results.[42] Thiazolidinediones (TZD) including troglitazone and rosiglitazone, which are peroxisome proliferator-activated receptor-γ (PPARγ) ligands, have been shown to potently stimulate in vitro adipogenesis.[43-45] PPARγ has been identified as a master regulator of adipogenic differentiation.[46] In general, the supplemented factors must be present at physiological concentrations or higher to have an effect.[12]

## Transcriptional Control of Adipocyte Differentiation

The differentiation of human ASC involves a complex series of changes in the cellular gene and protein expression patterns.[1,47] In vitro differentiation is characterized by growth arrest, the induction and expression of multiple adipogenic genes (Fig. 1), and ultimately, triglyceride accumulation.[48] Three groups of transcription factors have been identified as key regulators[1] of adipocyte differentiation: (1) PPARγ,[49] (2) the CCAAT/enhancer binding proteins (C/EBP)[50] and (3) adipocyte determination- and differentiation-dependent factor-1/sterol regulatory element-binding protein-1 (ADD-1/SREBP-1).[51] Figure 2 highlights some of the interactions of these key factors. Detailed reviews of the transcriptional cascades can be found in the literature.[52-55]

## Adipose Tissue Engineering

To date, a number of different constructs have been investigated for adipose tissue-engineering applications. The general strategy

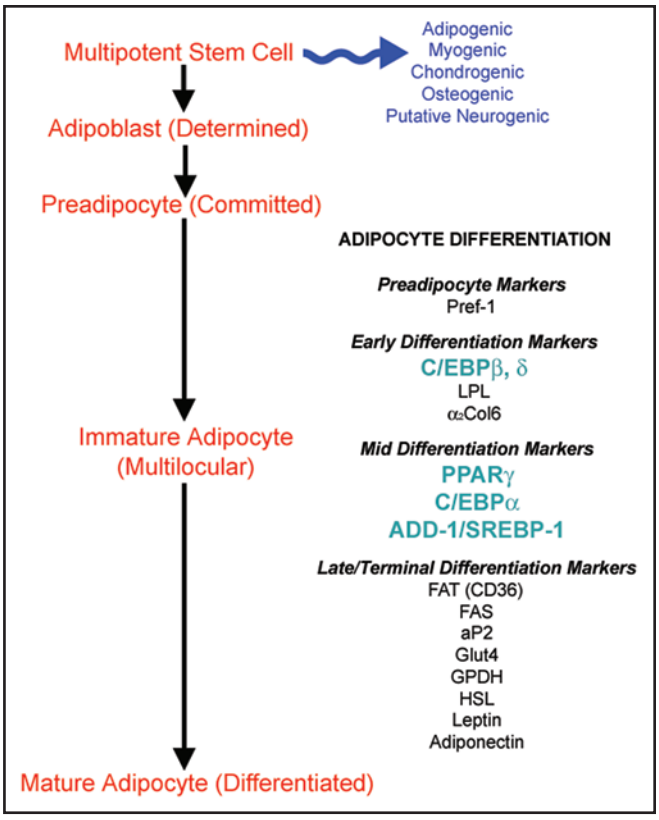


Figure 1. Overview of the differentiation of human ASC into mature adipocytes. The detectable markers associated with the various stages of differentiation are highlighted (LPL = lipoprotein lipase; $\alpha_2$Col6 = alpha 2 chain of collagen type VI; FAT = fatty acid translocase; FAS = fatty acid synthase; aP2 = fatty acid binding protein; Glut4 = glucose transporter-4; GPDH = glycerol-3-phosphate dehydrogenase; HSL = hormone sensitive lipase).

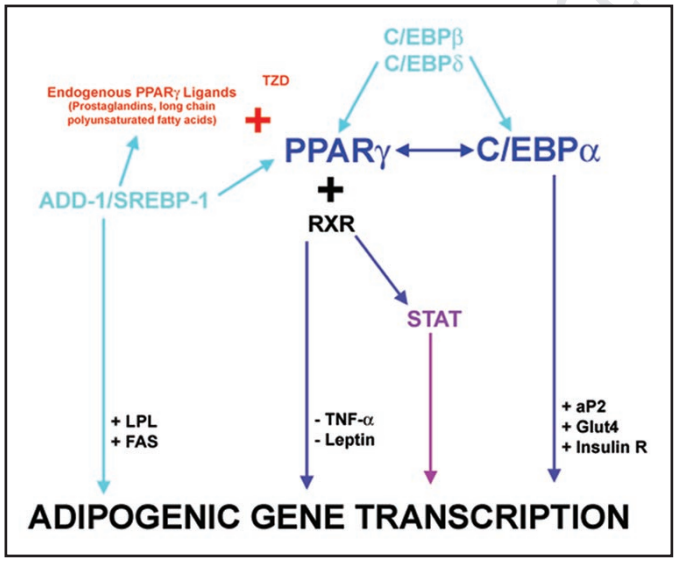


Figure 2. Transcriptional events in adipogenic differentiation. The diagram highlights the critical role of multiple transcription factors in adipogenesis. Arrows generally indicate a stimulatory interaction (with the exception of TNFα and leptin). After ligand binding with either endogenous ligands or TZD, PPARγ binds as a heterodimer with retinoid X receptor (RXR) to PPAR response elements (PPRE) in the transcriptional regulatory regions of the target genes. Insulin R., Insulin Receptor.

involves seeding an extracellular matrix (ECM) substitute with cells capable of contributing to the regenerative response. These studies have shown the promise of soft tissue regeneration. Further research is required to understand the optimal characteristics to facilitate stable fat formation.

## Cellular Component

Most tissue-engineering strategies involve preadipocyte cell lines or primary adipogenic stem cells. These approaches circumvent the limitations of mature adipocytes, which are large, fragile, and highly prone to ischemic cell death.[56] Further, terminally-differentiated adipocytes do not proliferate in their mature form, thereby limiting their regenerative potential.[9,10]

**Cell lines.** The most investigated adipogenic cell lines are the 3T3-L1, 3T3-F442a and Ob17 murine cell lines.[57,58] Clonally derived cell lines are homogeneous, well defined, and can be cultured in vitro for extended time periods. Cell lines circumvent many of the problems associated with primary cells including tissue availability and donor variability.[21,59] However, the primary cell response may be more representative of the true in vivo cellular behavior.[12] There are significant differences in the hormonal requirements for differentiation between cell lines and primary cells.[60,61] In general, cell lines differentiate much more readily than primary cells, and can spontaneously convert into adipocytes in the presence of serum if growth arrest is maintained through confluence.[62]

**Mesenchymal stem cells.** Bone marrow (BM) is a source of MSC capable of adipogenic differentiation.[63] However, BM procurement can be painful and the cell yield is often extremely low, with only a very small percentage (0.001–0.01%) of the cells having multilineage potential.[64,65] The culture conditions must be carefully regulated, and serum selection and growth factor supplementation are commonly required to facilitate expansion.[64] The differentiation capacity tends to decrease with increasing passage and time in culture.[66]

As discussed, adipose tissue is another possible MSC source for tissue engineering. Most patients are able to donate sufficient fat for cell isolation without any adverse effects.[5,56,67] Using centrifugal separation and filtration techniques, up to 1 x $10^8$ viable ASC can be isolated from processed tissues in the range of 100–500 mL.[68] ASC are more resistant to mechanical damage and ischemia than mature adipocytes.[1,69] During lipogenesis, differentiating ASC synthesize new ECM and reorganize the matrix into the mature form found in fat.[70] Hence, ASC may remodel tissue-engineered constructs to more closely resemble native adipose tissue ECM. ASC also secrete bioactive factors when differentiating that stimulate endothelial cell growth and motility in vitro and angiogenesis in vivo.[71-73] In immunosuppressed rodents, injected ASC have been shown to hone to sites of injury, engraft and contribute to regeneration.[63,65,74]

It is important to note that cell isolation techniques and culture methods dramatically impact ASC proliferation and differentiation. While the cells can be expanded in culture for extended periods of time, they will senesce.[75] The differentiation capacity also declines with increasing passage and freezing.[36,40] Differences in differentiation have been noted depending on the cell donor and the anatomic site of harvest.[76] Cells harvested from younger donors generally have a greater potential to proliferate and differentiate than those harvested from older donors.[41,42]

## Engineered Extracellular Matrices

A tissue-engineered adipose substitute should facilitate complete and predictable volume restoration.[1] As mentioned, a common strategy is to incorporate a 3D scaffold that defines and maintains the desired tissue volume. The constructs must be easy to handle in the clinic and be readily modifiable to suit the individual needs of each patient.[77] These ECM substitutes should facilitate normal cellular organization and behavior, promote regeneration, and restore functionality.[1] Materials should be selected that are capable of controlled, non-toxic degradation, as they are replaced by healthy host tissues. Some of the results to date for a variety of investigated scaffolds are reviewed below.

**Synthetic scaffolds.** Poly(lactic-co-glycolic) acid scaffolds. Patrick et al.[78] designed poly(lactic-co-glycolic) acid (PLGA) scaffolds seeded with primary rat adipose precursor cells, harvested from epididymal fat pads. When implanted into the dorsa of rats, differentiated adipocytes could be detected within the constructs by 5 weeks. Longer term in vivo studies showed that the adipose tissue completely resorbed by 5 months, likely due to scaffold degradation.[77]

PLGA-polyethylene glycol (PLGA-PEG) microspheres were studied as drug delivery vehicles for insulin, insulin-like growth factor-1 (IGF-1), and basic fibroblast growth factor (bFGF) by Shenaq and Yuskel.[79] In a subcutaneous rat model, incorporating the growth factors improved autologous free fat graft weight and volume, with the best results observed for either insulin or IGF-1 alone or in combination. A PLGA (75:25) foam was also assessed in vivo in combination with IGF-1 and insulin, with fibroelastic tissue formation at the implantation site at 12 weeks.

In another study evaluating the impact of bFGF, Neubauer et al.[80] investigated rat BM MSC on PLGA scaffolds in vitro with or without the addition of bFGF. The investigation, which included measurements of GPDH activity and PPARγ and Glut4 gene expression, indicated that bFGF had a positive effect on adipogenesis.

Polyglycolic acid scaffolds. The cellular response of 3T3-L1 cells induced to differentiate in vitro on polyglycolic acid (PGA) scaffolds was investigated by Fischbach et al.[81] Within the scaffolds, the cells readily accumulated lipid and assumed a mature, unilocular morphology by 35 days in culture. Interestingly, leptin secretion and laminin expression were augmented in comparison to traditional two-dimensional culture. When the constructs were subcutaneously implanted in nude mice, histological results showed the formation of mature fat pads surrounded by a fibrous capsule at 35 days.

Polytetrafluoroethylene scaffolds. The differentiation of human ASC on non-degradable polytetrafluoroethylene (PTFE) surgical mesh coated with collagen, albumin or fibronectin was examined by Kral and Crandall.[67] While all of the coatings improved cell adhesion compared to the uncoated control, the highest seeding efficiency was observed with the fibronectin coating. Differentiation was induced, and some lipid accumulation was reported using SEM at 2 weeks after the induction of differentiation.

Poly(ethylene glycol)-diacrylate hydrogels. Alhadlaq et al.[82] reported on the encapsulation of human BM MSC within poly(ethylene glycol)-diacrylate (PEGDA) hydrogels. Differentiation was assessed at 4 weeks after seeding both in vitro and in vivo (subcutaneous athymic mouse model). In vivo, the scaffolds were shown to maintain their architecture and immunohistochemical staining indicated that the encapsulated cells expressed PPARγ. A later study confirmed that the cell-seeded PEGDA scaffolds facilitated adipose tissue formation and had excellent volume retention properties after 4 weeks in vivo.[83] More specifically, the scaffolds showed 100% volume retention as compared to 35–65% volume retention for collagen gels within the same system.

Polyethylene terephthalate scaffolds. The in vitro response of 3T3-L1 cells seeded on non-degradable polyethylene terephthalate (PET) scaffolds was characterized by Kang et al.[84] Oil red O staining showed high levels of intracellular lipid accumulation, with most of the cells having a mature morphology by 14 days after the induction of differentiation. RT-PCR analysis showed that the cells expressed multiple adipogenic markers including PPARγ, leptin, aP2 and Glut4. Similar to the results with the PGA scaffolds, the cells were shown to secrete higher levels of leptin in the three-dimensional scaffolds as compared to two-dimensional controls.

**Naturally derived scaffolds.** Matrigel™. Matrigel™ is a commercially available product that is derived from the reconstituted basement membrane of mouse sarcoma. This gelatinous material contains an undefined mixture of proteins, including collagen type IV, laminin and a variety of growth factors. Matrigel™ has been shown to support cell growth and has been used extensively to study cellular responses within a 3D environment. However, its clinical application is prohibited by its murine and tumorigenic origins.[85]

Kawaguchi et al.[86] demonstrated de novo adipose tissue formation when Matrigel™ was subcutaneously injected with bFGF in nude mice. The material induced a strong neovascularization response within 1 week, followed by the recruitment and differentiation of endogenous precursor cells. The fat pad was reported to have expanded for the first 3 weeks after implantation and retained its volume for the entire 10-week study. Matrigel™ and bFGF also supported in vivo adipogenesis in combination with seeded 3T3-F442A cells.

Matrigel™ scaffolds containing bFGF were also investigated in vivo within a specialized silicone housing that isolated a pedicled blood supply by Walton et al.[87] The authors noted significant adipogenesis and the maintenance of volume when both Matrigel™ and bFGF were implanted. Fibrovascular tissue growth and the formation of a fibrous capsule were also noted. A similar study was reported by Vashi et al.[85] Collagen was used in place of Matrigel™ and FGF-2 was encapsulated in microspheres to facilitate long-term delivery. Histological results indicated that the collagen combined with FGF-2 microspheres could facilitate adipose tissue formation, although a fibrous capsule could be observed surrounding the implant.

Collagen scaffolds. Collagen scaffolds have been extensively studied as cell adhesive biomaterials for a variety of tissue engineering applications. Collagen has advantages in terms of availability, cost, biodegradability, and relative biocompatibility.[88,89]

Von Heimburg et al. seeded collagen scaffolds with human ASC and subcutaneously implanted them in nude mice for 3 and 8 weeks.[90] The implants were generally coated in a thin layer of adipose tissue that contained new blood vessels. Under histological analysis, adipose tissue formation and neovascularization of the outer layers of the construct were confirmed. A fibrous capsule was reported and there was a significant decrease in graft thickness and structural collapse with time.

Gelatin microspheres containing bFGF were combined with human ASC and collagen sponges by Kimura et al. and the constructs were implanted in nude mice.[91] Adipose tissue developed in the constructs within 6 weeks. All three components were required to induce the response, and the extent of adipose tissue formation was dependent on the number of seeded cells and the bFGF concentration. It was hypothesized that the bFGF may have contributed to adipogenesis by promoting angiogenesis.

Gentleman et al.[92] investigated collagen gels embedded with short collagen fibres to make the constructs more resistant to cellular contraction. The gels were seeded with 3T3-L1 cells. At 21 days after the induction of differentiation, the cells were shown to have accumulated intracellular lipid.

Glutaraldehyde cross-linked collagen-chitosan hydrogels for soft tissue augmentation were developed by Wu et al.[93] The scaffolds were seeded with primary rat adipose precursors isolated from the epididymal fat pad. In vitro, the cells could be visualized on the scaffolds and were viable over a 10-day period. In a subcutaneous rat model, Oil Red O positive cells were present in the scaffold at 7 and 14 days.

HYAFF® scaffolds. A group in the Department of Plastic and Reconstructive Surgery at the Aachen University of Technology has extensively studied biodegradable HYAFF® scaffolds for adipose tissue engineering applications. HYAFF® is a semi-synthetic material that is a benzyl ester of hyaluronan (HA). In 2001, they investigated HYAFF® sponges, HYAFF® nonwoven carriers and freeze-dried collagen scaffolds, seeded with human ASC in a subcutaneous athymic mouse model.[94] The results indicated that the strongest differentiation response was observed within the pores of the HYAFF® sponges. Histology showed the presence of clusters of differentiating cells within the sponges at 3 weeks. In contrast, a greater number of undifferentiated ASC were observed in the nonwoven carriers. In 2003, they investigated cellular adhesion of human ASC on HYAFF® scaffolds with varying degrees of esterification (i.e., hydrophobicity) and pore size.[95] The results indicated that the greatest adhesion was observed with the scaffolds with 100% esterification and a mean pore size of 400 μm. Differentiation was observed when the scaffolds were maintained in adipogenic culture. In 2005, the group reported on a new 400 μm pore HYAFF® sponge with a different architecture.[96] In addition, they investigated the effect of coating the scaffolds with HA to make the constructs more hydrophilic and allow greater infiltration of the aqueous cell suspension. The scaffolds were seeded with human ASC and implanted for 3, 8 and 12 weeks in a subcutaneous athymic mouse model. The scaffolds showed improved cellular penetration and neovascularization as compared to the previously studied HYAFF® sponges. However, histological analysis indicated that there was only minimal adipose tissue formation within the constructs.

Alginate scaffolds. Marler et al.[97] investigated injectable alginate scaffolds seeded with syngeneic fibroblasts in an inbred rat subcutaneous model. A modified form of alginate was developed that incorporated the cell-adhesive peptide arginine, glycine and aspartic acid (RGD). A greater loss of volume was reported in the unseeded RGD-modified scaffolds (25% volume retention at 8 weeks) as compared to the unmodified controls (58% volume retention at 8 weeks). The addition of the fibroblasts improved the volume retention in both gels. In general, a fibrous capsule could be observed surrounding the implanted material. The RGD-modified material facilitated fibrovascular ingrowth from the surrounding tissues, which resulted in the fragmentation of the gel. Adipose tissue formation was not reported within any of the constructs.

Fibrin scaffolds. Injectable fibrin matrices containing human ASC and bFGF were investigated by Cho et al. in an athymic mouse model.[98] The gel was injected within an implanted PGA fibre mesh dome, reinforced with poly(lactic acid) (PLA). This supporting structure preserved the original volume of the implant for a 6-week period. Oil Red O staining indicated that there was adipose tissue formation within the fibrin gel when the dome was used.

Borges et al.[99] investigated the angiogenic potential of human ASC in fibrin glue in a chorioallantoic membrane model. The study indicated that the ASC were required to facilitate vascularization. Interestingly, the addition of vascular endothelial growth factor (VEGF) and bFGF to the medium did not influence the extent of vascularization.

Decellularized matrices. Our adipose tissue engineering research has focused on the investigation of naturally derived placental decellular matrix (PDM) and cross-linked hyaluronan (XLHA) scaffolds for soft tissue augmentation. Our initial work involved the development of a protocol to decellularize an intact, large segment of the human placenta.[100] Following the finalization of the decellularization protocol, the in vitro cellular response of primary human ASC to several different PDM and XLHA scaffolds was studied.[101,102] Cellular organization, viability, proliferation, and glucose consumption were assessed during the proliferative phase prior to the induction of differentiation. The ASC differentiation response was characterized in terms of GPDH enzyme activity, intracellular lipid accumulation, and adipogenic gene expression using end-point (LPL, PPARγ, aP2, Glut4, leptin, GPDH) and real-time RT-PCR (PPARγ, GPDH). The cellular response was impacted by the scaffold environment and cell donor source. The cell-adhesive PDM scaffolds promoted ASC attachment, spreading and proliferation. In contrast, while all of the scaffolds could support the adipogenic differentiation of the ASC to some extent, differentiation was augmented when the cells were encapsulated in the non-cell-adhesive XLHA gels.

The PDM and PDM with XLHA scaffolds, seeded with primary human ASC, were also investigated in a subcutaneous athymic mouse model.[103] The in vivo response at 3 and 8 weeks was characterized using histological and immunohistochemical staining. The relative number of adipocytes within each implant was quantified. Undifferentiated ASC were localized using immunostaining for human vimentin. Unilocular and multilocular adipocytes were identified by Oil Red O staining and implant vascularization was assessed by staining for murine CD31. The 3D volume of the PDM and PDM with XLHA implants was qualitatively maintained over the course of the study. Mature adipocytes were detected within both types of scaffolds, generally in greater numbers at the 8-week time point. There were extensive numbers of adipocytes within a number of the implants, with the developing tissues closely resembling normal adipose tissue. Incorporating the XLHA appeared to have a positive effect on angiogenesis and adipogenesis.

## Vascularization

To facilitate large volume soft tissue regeneration, it is important to recognize that adipogenesis and angiogenesis are highly

interconnected processes.[104-106] Following implantation, tissue-engineered adipose substitutes must rely on either revascularization or inosculation to become incorporated into the host vasculature.[1] Revascularization is limited by the slow kinetics of nutrient and waste diffusion. This process may be insufficient to support cellular survival in large or dense matrices.[56] In such devices, it may be necessary to create a capillary network within the adipose substitute and subsequently fuse this engineered system with the host vasculature.[107]

## Summary and Future Outlook

Adipose tissue engineering studies to date have shown that regeneration is possible using a cell-seeded scaffold approach. As discussed, many different types of scaffolds have been studied both in vitro and in vivo. Every scaffold has associated strengths and limitations, and the ideal environment for stable fat formation over the long-term remains unresolved.

While early research focused on qualitative histological assessments, the application of cellular and molecular biology techniques is required to develop a more thorough understanding of the cellular behavior. Techniques such as end-point and real-time RT-PCR, flow cytometry, Western blotting and DNA and cytokine arrays, should be used to characterize the gene and protein expression patterns of cells seeded within the scaffold environments. By using these approaches, it may be possible to develop a better understanding of the scaffold properties that promote adipogenesis, thereby facilitating construct optimization.

Human ASC may represent an ideal cellular population for a range of tissue-engineering applications, including adipose tissue regeneration. As discussed, the investigation of primary human cells may provide more relevant data to facilitate the ultimate goal of clinical application. The ASC population includes multipotent mesenchymal stem cells capable of differentiation along multiple lineages.[108,109] To date, most adipose tissue engineering studies involving cell-seeded scaffolds have focused solely on probing adipogenic markers. However, to understand the impact of the various scaffold environments on the cellular behavior, the key signal transduction pathways involved in the commitment process should be probed. MSC express factors from each of the lineages, which cross-regulate one another and maintain the undifferentiated state.[54] During differentiation, there is an increase in the key transcription factors from one specific lineage, combined with a repression of the controlling factors for the other lineages. It is recognized that cellular interactions with scaffolds can influence signal transduction.[110] As such, it is likely that there are scaffold properties that would favor adipogenic differentiation of seeded MSC.

Our research has suggested a possible role for cell adhesion and shape in the modulation of adipogenic differentiation.[101,102] Primary adipocytes maintain their mature, unilocular phenotype when cultured in suspension. When promoted to adhere to a surface, the adipocytes spread and de-differentiate.[111] Spiegelman and Ginty showed that adipogenic differentiation in the 3T3-F422A cell line was inhibited when strong cellular adhesion to fibronectin matrices was promoted, while disrupting the actin cytoskeleton and culturing the cells in a rounded state could restore the differentiation capacity.[112] Cell shape was also shown to modulate human MSC differentiation through the RhoA-ROCK signalling pathway, with adipogenic differentiation favored in MSC that were cultured with a rounded phenotype. In contrast, osteogenic differentiation was observed in culture conditions that promoted cellular attachment and spreading.[113] These results could have implications on the success of cell-seeded scaffolds for adipose tissue engineering.

Overall, adipose tissue engineering is an exciting and emerging field. By building on the work conducted to date, the ultimate goal of predictable, long-term soft tissue regeneration for volume augmentation may be possible in the future.



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